ANTIBODY RESPONSE TO REPEATED TRANSFUSIONS IN COMPATIBLE CANINE RECIPIENTS

David E. Meixner, et al

Army Medical Research Laboratory
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SP6 David E. Meixner, COL Charles E. Shields, MC (M.D.), and Angelo I. Zegna

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13. ABSTRACT

Several apparent transfusion reactions were observed in a group of experiments involving multiple nonautologous transfusions in dogs. Prior to the transfusions, crossmatches between donor and recipient were performed, which were compatible in most of the dogs. The canine grouping system formulated by Swisher and Young was used to group most of the dogs. Red cell survivals were determined using ^{51}Cr. A rapid loss of erythrocyte radioactivity after transfusion was considered to be evidence of incompatibility. Efforts to determine the source of the incompatibility suggested that the present canine blood group system does not provide sufficient knowledge of the factors involved. The incompatible reactions also suggested the presence of other antigens not defined by Swisher and Young's system. The use of multiple transfusions in dog experiments--in which results depend upon red cell findings--may cause confusion because of unsuspected blood incompatibility reactions.

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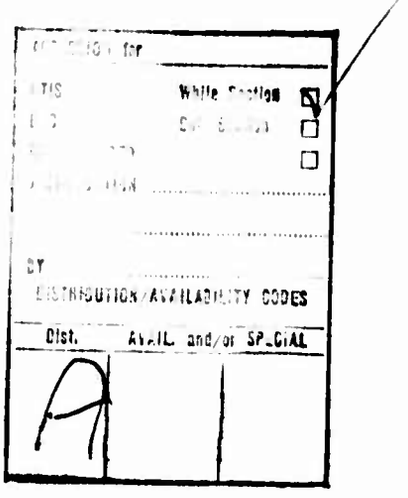

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ANTIBODY RESPONSE TO REPEATED TRANSFUSIONS IN COMPATIBLE CANINE RECIPIENTS

(Progress Report)

by

SP6 David E. Meixner
COL Charles E. Shields, MC (M.D.)
and
Angelo I. Zegna

US ARMY MEDICAL RESEARCH LABORATORY
Fort Knox, Kentucky 40121

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ABSTRACT

ANTIBODY RESPONSE TO REPEATED TRANSFUSIONS IN COMPATIBLE CANINE RECIPIENTS

OBJECTIVE

To demonstrate incompatible transfusion reactions in dogs and their effect on red blood cell research.

METHODS

In a series of experiments involving multiple nonautologous transfusions in dogs, several apparent transfusion reactions were observed. Crossmatches between donor and recipient performed prior to the transfusion, using standard crossmatching technique, were compatible in most of the dogs. Red cell survivals were determined using ^{51}Cr. Most of the dogs were grouped according to the system formulated by Swisher and Young.

CONCLUSIONS

A rapid loss of erythrocyte radioactivity after transfusion was considered to be evidence of a reaction of blood incompatibility. Efforts to determine the source of the incompatibility suggested that the above blood grouping system does not provide sufficient knowledge of the factors involved. In addition, the incompatible reactions suggest that there are more blood cell antigens than those defined by the existing system.

While crossmatch procedures may be of some help to the clinician in determining gross incompatibility, the response of isotope-labeled erythrocytes may be a better method to detect incompatibility.

The use of multiple transfusions in dog experiments--in which results depend upon red cell findings--may cause confusion because of unsuspected blood incompatibility reactions.

ANTIBODY RESPONSE TO REPEATED TRANSFUSIONS IN COMPATIBLE CANINE RECIPIENTS

INTRODUCTION

In a series of experiments involving multiple nonautologous transfusions in dogs, several apparent transfusion reactions were observed. Crossmatches between donor and recipient performed prior to the transfusions were compatible in most of the dogs. On crossmatch testing, there were some minor side incompatibilities representing recipient cells which were apparently reacting to donor plasma antibodies. Since the experiment involved isotope labeling of donor cells, these recipient cell reactions would be undetected and were ignored for the purposes of this experiment. Most of the dogs were grouped according to the blood grouping system formulated by Swisher and Young (5), which will be referred to in this report as the "ACF system."

METHODS

Blood (100 ml) was drawn from selected dog donors and stored for periods of 14, 28, or 42 days in acid-citrate-dextrose (ACD) or ACD-adenine. Prior to each transfusion, each unit was tagged with 30 μCi ^{51}Cr and the units split so that two recipients received 50 ml each. The blood was infused at a rate of 10 ml per min. A total of 48 nonautologous transfusions was given to 25 beagles and seven mongrels. After the infusions, blood samples were drawn within 2 min, and at 15 and 20 min. Follow-up samples were taken daily until at least five samples had been obtained. Radioisotope levels were determined by using a well-type nuclear counter (Packard Auto-gamma spectrometer). Individual samples from the same dog were counted at the same time to adjust for decay. Volumes of red cells were adjusted with hematocrit. Red cell survivals were calculated as a percentage of the 2 min sample radioactivity, and plotted on semilog graph paper.

The pretransfusion crossmatches were performed using a standard crossmatching technique (8), with some modifications. It was found that saline suspended dog erythrocytes would hemolyze at 37 C so a 22 C incubation phase was used. For the other test, erythrocytes were suspended in their own serum and incubated at 37 C. An antiglobulin Coombs phase was performed (4) and all tubes were examined both macroscopically and microscopically. Both major and minor side crossmatches were performed.

While most of the pretransfusion crossmatches were compatible, the possibility of a sensitization and subsequent reaction caused by a blood type system incompatibility was recognized. When antisera to the ACF system was available, most of the dogs in the colony, including those already used in this experiment, were grouped. Not all incompatible crossmatch reactions could be related to difficulties in the ACF system, suggesting other blood systems may have been responsible.

RESULTS

None of the transfused dogs had clinical symptoms, however, some animals had an obvious loss of red cell radioactivity after transfusion, which was considered evidence of incompatibility between recipient and donor cells. Severe and rapid decrease in radioactivity with a T1/2 of less than 2.50 days was considered to be primarily a reaction to a transfusion incompatibility. Minor decreases of a T1/2 of less than 4.75 days but more than 2.50 days may have indicated a reaction but could not be established because of other experimental variables. Values of T1/2 greater than 4.75 days were accepted as normal. All animals demonstrating a T1/2 of less than 2.50 days were recrossmatched following the transfusion. Those recipients whose pretransfusion crossmatches were normal (negative) were found to have converted to a positive crossmatch, and the posttransfusion crossmatches of those recipients who were previously positive were found to exhibit a much stronger reaction.

The recipient dogs used in this experiment, results of their pretransfusion crossmatches, compatibility of ACF grouping, radioisotope percentages expressed as T1/2, and number of times each dog had been previously transfused are shown in Table 1. Note that several dogs are listed more than once and, in most of these cases, they had a different donor, so that on repeat testing, there was often a change in compatibility and reaction to the blood.

TABLE 1

RECIPIENT DOGS USED IN EXPERIMENT

Study No.	Crossmatch*	ACF Typing**	T1/2
	Dogs with no prior transfusions		
19		N	2.40+
20		A_2	3.30
23		N	4.86
	Dogs with one prior transfusion - Compatible crossmatch		
1		N	2.00+
2		F	0.50+
4		N	4.33
5		A_2	
6		A_2	4.65
7		A_2	4.86
8		N	4.65
9		--	5.50
10		Tr	6.75
13		N	7.40
14		A_1	6.60
17		A_2	5.10
18		A_2	5.10
21		--	5.40

TABLE 1 (cont)

Study No.	Crossmatch*	ACF Typing**	T1/2
22		--	2.00+
24		--	3.60
25		N	5.65
29		--	5.00
38		--	7.25
42		He	4.85
45		--	8.15
Dogs with one prior transfusion - Incompatible crossmatch			
3	3+	N	4.47
30	±	D,Tr	6.35
36 (=23)***	1+	B	4.20
37	2+	--	2.00+
39 (=19)	3+	--	2.00+
40 (=20)	4+	--	4.80
Dogs with two prior transfusions - Compatible crossmatches			
11 (=3)		N	5.75
12 (=8)		Tr	3.60
15 (=8)		N	2.50+
16 (=4)		A_1	6.20
33		--	6.90
34		A,B,D	6.20
35 (=24)		B,D	3.45
41		D,He	8.00
43 (=20=40)		A_2,D,He	4.15
46		N	7.75
47 (=19=39)		He	2.50+
Dogs with two prior transfusions - Incompatible crossmatches			
26	±	A_2	4.33
48 (=23=36)	4+	N	2.00+
Dogs with three prior transfusions - Compatible crossmatches			
27 (=3=11)		N	5.25
28 (=4=16)		N	0.04+
31 (=7=15)		A_2,He	5.25
32 (=8=12)		D,Tr	5.00
Dogs with three prior transfusions - Incompatible crossmatches			
44 (=24=35)	2+	D	3.90

*Degree of incompatibility indicated by grading of agglutination from ± to 4+.

**Blood group showing possible reactive groupings. Those without a listing were not done. N is normal, or compatible.

***The number in parentheses ( ) indicates a previous transfusion and that same dog being used as a recipient again.

+Excessive low T1/2 values indicative of a reaction. See text.

A summary of the effects of the transfusions is shown in Table 2. A comparison is made between the number of reactions occurring in dogs found to be compatible or incompatible by crossmatch (major side), or the ACF system.

TABLE 2

A SUMMARY OF THE EFFECTS OF TRANSFUSION

CROSSMATCHES	Compatible	Incidence of Reaction	Incompatible	Incidence of Reaction
No reaction	32		6	
Reaction	6	17%	3	30%
TOTAL	38		9	

ACF SYSTEM GROUPING	Compatible	Incidence of Reaction	Incompatible	Incidence of Reaction	Not Tested	Incidence of Reaction
No reaction	9		21		9	
Reaction	4	30%	2	10%	3	25%
TOTAL	13		23		12	

Chi square analysis of the two systems showed a high significance ($p < .001$) between systems, and the different incidence of reactions. The large group of theoretical incompatible transfusions was not accompanied by a corresponding high incidence of reaction.

Correlation was not found between the incompatible transfusion reactions and ACF system incompatibilities. Correlation between prior sensitization and the occurrence of reactions could not be established, usually because the same donor was not involved. Of those dogs exhibiting reactions, five had received one previous transfusion, three had been given two previous transfusions, and one had three previous transfusions. Some dogs exhibited no reaction when transfused with blood shown to be incompatible by crossmatch, even after more than one transfusion by the same incompatible donor. However, many of those incompatible by crossmatch did have a reaction.

DISCUSSION

A rapid loss of erythrocyte radioactivity after transfusion was considered to be evidence of a reaction of blood incompatibility. Efforts

to determine the source of the incompatibility suggested that the present ACF blood type system does not provide sufficient knowledge of the factors involved. These observations follow earlier work involving dog blood typing showing the ability of the canine immunological system to become sensitized and to react to subsequent antigens (1,2,7,8). In addition, the incompatible reactions suggest that there are more blood cell antigens than those defined by the ACF system.

Since crossmatch procedures which indicated positive reactions were more often associated with posttransfusion cell loss and/or converted to positive after such an incompatible reasponse, this test may still be of some assistance in detecting gross incompatibility. It is apparent, however, that the response of the isotope labeled cells may be a better method to detect incompatibility (3,9).

The ACF system had a larger number of theoretically incompatible transfusion situations than the crossmatch system, but these were not accompanied by a corresponding increase in detectable reactions. Such a reduction in the predictive value for the ACF system emphasized the need for pretransfusion crossmatch. However, crossmatch procedures were also subject to considerable error.

This work emphasized that the use of multiple transfusions in dog experiments--in which results depend upon red cell findings--may cause confusion because of unsuspected blood incompatibility reactions. Neither pretransfusion test system was adequately predictive, and the subjects rarely showed clinical signs--hence, the detection of rapid red cell destruction was left primarily to isotope survival studies.

SUMMARY

Several apparent transfusion reactions were observed in a group of experiments involving multiple nonautologous transfusions in dogs. Prior to the transfusions, crossmatches between donor and recipient were performed, which were compatible in most of the dogs. The canine grouping system formulated by Swisher and Young was used to group most of the dogs. Red cell survivals were determined using ^{51}Cr.

A rapid loss of erythrocyte radioactivity after transfusion was considered to be evidence of incompatibility. Efforts to determine the source of the incompatibility suggested that the present canine blood group system does not provide sufficient knowledge of the factors involved. The incompatible reactions also suggested the presence of other antigens not defined by Swisher and Young's system.

The use of multiple transfusions in dog experiments--in which results depend upon red cell findings--may cause confusion because of unsuspected blood incompatibility reactions.

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